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Application of Ionizing Radiation for Control of *Salmonella* in Food

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Abstract

Ionizing radiation (gamma rays from the radionuclides cobalt-60 or cesium-137, e-beams) is an effective, nonthermal method to reduce or eliminate food-borne pathogens, including *Salmonella* spp. both in raw and in cooked meats, poultry, fish, and shellfish. Irradiation treatment, applied as the final processing step, seems to be particularly promising in the case of packed food products, including ready-to-eat food. Final packaged food products can be contaminated from post-lethality exposure, that is, after heat treatment and before packaging. The application of ionizing radiation after packaging can eliminate or considerably reduce both saprophytic and pathogenic microflora in final products. It is of particular importance in the case of ready-to-eat food which is not subjected to heat treatment before consumption. According to hurdle concept technology, the combination of existing and novel preservation methods can ensure safety of food by applying all treatments as mild as possible. Irradiation treatment can be combined with the use of natural antibacterial compounds, such as extracts of spices and herbs, or various packaging systems. Doses of ionizing radiation required for the inactivation of *Salmonella* spp. in fish and seafood are lower than those used for meats and poultry.

Keywords: *Salmonella*, radiation, meat, poultry, fish

1. Introduction

In the last years, the great consumer interest in “natural” or “fresh” foods, nonprocessed or only minimally processed, has caused an increasing interest in nonthermal preservation methods, that is, ionizing radiation, ultraviolet radiation, high-pressure processing (HPP), pulsed electric field (PEF), high-pressure carbon dioxide (HPCD), the use of natural antibacterial compounds, such as extracts of spices and herbs, or the application of various packaging

systems. However, at the same time consumer demand for ensuring food safety has to be met. Those two ideas are very often tough to reconcile in practice.

A great number of studies have shown that ionizing radiation improve the safety of various foods of animal as well as plant origin. Food irradiation is a process which can be used to inactivate both food-borne pathogens and microorganisms causing spoilage of food, thus extending storage of foods such as red meats, poultry, fish, and so on. It can also extend the storage of vegetables by prevention of sprouting (potatoes, onions, and garlic) or fruits by the delay of ripening. At present time, this technology may be used not only to raw foods but also as post-lethality treatment. The product may be exposed to the post-lethality processing environment into which the product is routed after having been subjected to an initial lethality treatment. The foodstuffs may be exposed to the environment in the area of establishment as a result of, for example, slicing, peeling, and re-bagging, or other procedures. Hotdog products are examples of ready-to-eat (RTE) meat and poultry products that receive a lethality treatment to eliminate pathogens (core temperatures of +70° to +72°C must be reached due to cooking) and they are subsequently exposed to the environment during peeling, slicing, and repackaging operations. Then, the technology of irradiation, used as an intervention step, can be applied to the final product or sealed package of product in order to reduce or eliminate the level of pathogens resulting from contamination from post-lethality exposure. Thus, for example, vacuum-packaged ready-to-eat (RTE) meat products may be subjected to irradiation to reduce or eliminate dangerous food-borne pathogens such as *Salmonella* spp. and *Listeria monocytogenes* in a final food product. According to hurdle concept technology, the combination of existing and novel preservation methods can ensure safety of food by applying all treatments as mild as possible [1, 2].

A good example of such combination of preservation methods (low-dose irradiation and modified atmosphere packaging (MAP)) is the work of Chouliara et al. [3] who investigated the combined effect of gamma irradiation (2 and 4 kGy) and modified atmosphere (MA) packaging (30% CO₂/70% N₂ and 70% CO₂/30% N₂) on shelf-life extension of fresh chicken meat stored under refrigeration. The authors noted the reduction of the number of various groups of bacteria (from 1 to 5 logs), including Enterobacteriaceae family. Sensory evaluation showed that the combination of irradiation at 4 kGy and MAP (70% CO₂/30% N₂) resulted in the highest shelf-life extension by 12 days compared to the air-packaged samples. A study of Grant and Patterson [4] is another good example of hurdle concept technology: mild heating combined with low-dose irradiation. In this study, thermal treatment (70, 65, or 60°C) was applied alone, directly post 0.8 kGy irradiation or post irradiation combined with refrigerated storage on inactivation of *L. monocytogenes* and *Salmonella typhimurium* inoculated into beef and gravy. The researchers observed heat sensitization of *S. typhimurium* at 60°C, but not at either 65 or 70°C like in the case of *L. monocytogenes*. In another study [5], the influence of heating and low-dose irradiation *S. typhimurium* in MDCM (mechanically deboned chicken meat) was examined. The researchers noted that salmonellae irradiated with 0.9 kGy were more heat sensitive; this effect was maintained during 6 weeks of refrigerated storage.

Those readers who want to deepen their knowledge of the subject can find an extensive description of microbiological issues associated with all muscle foods, their specific spoilage, safety issues, and their control for meat, poultry, and seafood in the work provided by Sofos et al. [6].

Thermal treatment is a very effective method for eliminating *Salmonella* spp. in foods. This organism is rather sensitive to pasteurization temperatures used in meat processing. Properly conducted heat treatment in industrial food processing should cause complete inactivation of these bacteria in meat and meat products; however, recontamination of ready-to-eat meat products with *Salmonella* spp. after cooking, as well as subsequent storage at abuse temperatures at food establishments or at a consumer's home, can cause a significant risk to human health. Szczawińska et al. [7] inoculated commercial, smoked, cured, and cooked ham with *Salmonella* enteritidis and stored the samples at abused temperature (15°C). Lag time for *S. enteritidis* was at that temperature only 139.08 h, that is, less than 6 days [7]. Usually, the length of time for storage of such product recommended by the food manufacturer is much longer than the time mentioned above. It means that a consumer can contract food-borne salmonellosis during the recommended length of storage time for such ready-to-eat meat product if it was recontaminated with *Salmonella*. Thus, due to beneficial effects of ionizing radiation treatment of final packaged food product (RTE), we can expect that *Salmonella* (and other vegetative bacterial pathogens which show similar radiation resistance, e.g., *L. monocytogenes*) will be significantly reduced or eliminated.

2. The use of ionizing radiation

According to the Codex General Standard for Irradiated Foods [8], the following sources of ionizing radiation can be used:

- (a) Radionuclides, such as Cobalt-60 and Cesium-137, which emit gamma rays (γ -rays)
- (b) Machines that produced high-energy electron beams (an energy level up to 10 MeV)
- (c) X-rays machines (an energy level up to 5 MeV).

Compared to γ -rays, e-beams are characterized by a low penetrative capacity; therefore, e-beam irradiation is particularly useful for products which can be processed in thin layers or surface-contaminated products.

The dose of radiation received is commonly measured in grays. One gray is a derived unit of ionizing radiation. It is defined as the absorption of one joule of radiation energy in a mass of one kilogram (1 Gy = 1 J/kg). The gray has superseded the older unit—the rad (1 Gy = 100 rad). The **gray** (symbol: **Gy**) is used as a measure of absorbed dose.

According to several objectives for food (fresh or processed meats, poultry, and seafood) irradiation, the following terms are used [9]:

- (a) **Radication** is the elimination of bacterial pathogens, non-spore formers; doses range 2.5–10 kGy.
- (b) **Radurization** is the significant reduction of the number of saprophytic microorganisms ensuring shelf-life extension of foods; doses range 0.75–2.5 kGy.

- (c) **Radappertization** is based on a similar concept (“botulinum cook”) like in canning industry. It should ensure complete elimination of spore formers in foods, thus significant shelf-life extension (years) and botulism food safety; doses range 30–40 kGy. The term was established to honor Nicolas Appert who invented the method of preserving food from spoilage by placing it in hermetically sealed containers and then sterilized by heat treatment.

In this review, special attention will be paid to **radicidation**. In case of this technology, one of the most important pathogens, *Salmonella* spp., public health problem, has been the main target for control, particularly in meat and poultry products (i.e., for example, see Ref. [10]). The most recent European Food Safety Authority (EFSA) summary report has informed us that the total number of food-borne outbreaks in Europe was 5251, including water-borne outbreaks [11]. *Salmonella* caused 20.0% of all reported food-borne outbreaks in European Union (EU) and it was the second most frequent cause of outbreaks; the largest number of reported food-borne outbreaks was caused by viruses (20.4% of all outbreaks) [11]. High level of noncompliance was noted for poultry meat [11]. Monitoring activities and control programs for *Salmonella* in fresh broiler meat are based on sampling at the slaughterhouse and/or at processing or cutting plants and at retail. In 2014, *Salmonella* was found in 0.6% of the 2263 units of RTE broiler meat products tested at retail or at processing (0.4% of single samples and 1.7% of batches) [11].

As in previous years, the two most commonly reported *Salmonella* serovars in 2014 were *S. enteritidis* and *S. typhimurium*, representing 44.4% and 17.4%, respectively, of all reported serovars in confirmed human cases [11]. Generally, there was no major change as regards *Salmonella*-contaminated foodstuffs compared with previous years. *Salmonella* was most frequently detected in fresh turkey meat (3.5%), fresh broiler (2.2%), pig (0.5%), and bovine meat (0.1%) [11]. It should be emphasized that according to the European legislation on microbiological criteria for foodstuffs [12] *Salmonella* spp. is currently included both in food safety as well as food hygiene criteria.

The main reason for the use of food irradiation is the ability of ionizing radiation to inactivate populations of microorganisms including pathogenic bacteria, parasites, and viruses. Depending on irradiation dose, food-borne pathogens can be injured or killed due to DNA damage. Radiation sensitivity depends on many factors such as species of microorganisms, age of cells, and their number. It is also affected by the environment (buffer solution, laboratory medium, or real food product). Thus, the effect of radiation on microorganisms is dependent on intrinsic and extrinsic factors which include temperature, water activity, pH, chemical composition, and structure of food and gaseous environment. Radiation resistance of bacteria is much higher at freezing temperatures than at chill temperatures; however, irradiation of frozen food offers much better results in some foods because it significantly reduces or eliminates some negative sensory changes caused by, for example, lipid oxidation. D10 values (D10 value is defined as decimal reduction dose or the dose of ionizing radiation required for a 90% inactivation of viable colony-forming unit (CFU) or by one logarithmic cycle) are higher in foods with a low water activity because the lack of water means that there are less OH radicals available to cause DNA damage. Hence, higher doses of ionizing radiation have to be used to ensure the elimination of pathogenic bacteria in dry foods such as spices [13].

Some authors observed different effects of meat irradiation depending on radiation source. Rajkowski et al. [14] discovered in their study that D10 values for *S. typhimurium* DT 104

irradiated in ground pork with gamma rays were 0.56–0.62 kGy, whereas D10 values for the same organism treated with e-beams ranged from 0.42 to 0.43 kGy.

However, Miyahara and Miyahara [15] concluded that both gamma rays and e-beams were similarly effective while irradiating ground beef patties inoculated with *S. enteritidis*.

The use of ionizing radiation as a means of reducing the risk to human health from food-borne pathogens, including *Salmonella* spp., is being extensively researched. It seems that the application of ionizing radiation to preserve food or eliminate pathogenic bacteria from food has been so intensively studied like not any other scientific field, because of consumer concerns, particularly associated with fear of nuclear energy and very often occurring confusion between terms, for example, radiation, radioactive contamination, or radioactivity. In general, consumer is rather reluctant to this technology due to well-known nuclear accidents (e.g., Chernobyl and Fukushima) believing that the process of food irradiation can make food radioactive, thus unsafe. Interestingly, there is much less consumer resistance to the high-pressure-processing technology which is used to treat wide range of foods including those of animal origin, for example, RTE products. To date, health and safety authorities in over 60 countries worldwide, for example, the United States, France, Belgium, the Netherlands, Canada, Australia, and New Zealand, granted clearances for irradiation of more than 60 different foods [16]. Frog legs are the most often irradiated food items [17].

Currently, the International Atomic Energy Agency (IAEA) is responsible for updating and maintaining various irradiation databases as resources for researchers, government officials, and the general public. European Food Safety Authority [18] summarized and evaluated an opinion on the efficacy and microbiological safety of irradiation of food taking into consideration recommendations from the two panels: BIOHAZ (the EFSA Panel on Biological Hazards) and CEF (the EFSA Panel on Food Contact Materials, Enzymes, Flavours and Processing Aids).

EFSA emphasizes its standpoint that food irradiation should only be used in conjunction with an integrated food safety management program. With regard to efficacy and microbiological safety, the BIOHAZ Panel recommended that the application of food irradiation should be based on risk assessment and on the desired risk reduction rather than on predefined food classes/commodities and doses [18]). Concerning the safety assessment of irradiation of food, according to the BIOHAZ Panel, there are no microbiological risks for the consumer linked to the use of food irradiation and its consequences on the food microflora. EFSA's experts conclude that the irradiation dose needed to inactivate food-borne pathogens depends on the targeted pathogen, on the reduction required, and on the physical state of the food, **regardless of the food classes** as previously proposed [18].

2.1. Reducing *Salmonella* spp. in red meats and poultry

Vegetative food-borne bacteria, such as *Salmonella* spp. and *L. monocytogenes*, are moderately sensitive to ionizing radiation. The medium-dose irradiation processes reduce their populations by several logs. As previously mentioned, various factors influence radiation sensitivity of bacterial cells. The presence of proteins can exert a protective effect on microorganisms subjected to radiation treatment. Maxcy and Tiwari [19] studied the effect of fat content in beef on radioresistance of *S. enteritidis*. They found D10 value higher in beef with lower level

of fat (0.70 kGy) compared to lower D10 value obtained for salmonellae irradiated in beef with higher content of fat (0.49 kGy). Assuming that the low fat level in the meat is correlated with a higher protein content and because the proteins have the properties of free radicals scavenging, it can be suggested that the higher content of protein in meat protects more the bacteria against the damaging effects of radiation treatment.

There have been frequently voiced concerns that the reduction of the competitive microflora by radiation treatment could facilitate growth of pathogens contaminating the food after irradiation or that food pathogens which survived irradiation can grow better than the indigenous, competitive microflora. Dickson and Olson [20] studied the first problem; ground beef was irradiated at 0, 2, or 4 kGy, thus reducing the number of saprophytic microorganisms which cause food spoilage, and then inoculated with a mixture of four serotypes of salmonellae. The meat was stored at 4°C, temperature proper for storage, and at two abused temperatures 15 and 25°C. Bacterial growth was monitored during storage. The authors observed that there was no significant difference in lag-phase duration or generation time, irrespective of the dose to which the ground beef had previously been exposed. This suggests that, although irradiation eliminates a significant portion of the spoilage microflora in ground beef, the absence of this microflora provides no competitive advantage to the growth of salmonellae in ground beef [20]. Szczawińska [21] studied the effect of irradiation on the survival rate of non-sporing bacteria (*Staphylococcus aureus*, *S. typhimurium*, *Escherichia coli*, *Pseudomonas fluorescens*) during conventional methods of meat preservation (heating, chilling, freezing, salting, curing, and smoking). On the basis of the conducted experiments, it can be concluded that irradiated bacteria stored under conditions preventing their growth die faster compared to unirradiated bacteria or their survival rate is almost identical like unirradiated ones; those organisms which are stored under conditions that allow their growth show a worse adaptability to the environment and begin to grow after a certain delay [21]. In another work, Szczawińska et al. [22] studied the growth of salmonellae in mechanically deboned chicken meat (MDCM), which was irradiated at 0, 1.25, and 2.5 kGy and inoculated with *S. dublin*, *S. enteritidis*, and *S. typhimurium*. Subsequently, the inoculated MDCM was stored at 5, 10, or 20°C and bacterial numbers were determined over storage time. The results of the study suggested that there was no greater risk from the same number of *Salmonella* cells contaminating irradiated MDCM compared to unirradiated one. In the same study, irradiated indigenous microflora had dose-related increased lag phases and decreased rates of multiplication compared with that of the indigenous microflora in the unirradiated control [22]. Kim and Thayer [23] discovered that the gamma-injured *S. typhimurium* cells on mechanically deboned chicken meat were much more sensitive to heat than the nonirradiated cells, which implies that any cells surviving the irradiation process were unlikely to survive cooking. This increased sensitivity of the salmonellae to gamma radiation was retained during refrigerated storage of the irradiated chicken. Kim and Thayer [23] explained the mechanism of the heat sensitivity of *S. typhimurium* subjected to ionizing radiation. The results proved that combined effects of irradiation and heating were always beneficial in regard to food safety due to synergistic (when heating is applied after irradiation) or additive (when heating is applied before irradiation) effects depending on the order of both treatments. Therefore, on the basis of these studies it can be concluded that any microorganisms which survive irradiation are more sensitive to intrinsic or extrinsic factors, such as temperature, water activity, pH, and so on, compared to unirradiated organisms.

Irradiation of fresh meat up to an overall average dose of 2 kGy was proposed by the SCF in 1986 [24]. Implication of meat in food-borne salmonellosis still remains a concern, particularly in the countries or regions where traditional dishes are served and consumed as raw and cold. In the Netherlands, Belgium, such meat product is “filet américain” composed of raw beef meat, and often raw egg. Kampelmacher [25] reported that a dose of only 1 kGy decreased *Salmonella* number in such a product by two log cycles. Rajkowski et al. [14] examined the effect of e-beam and gamma rays irradiation on the mixture of *S. typhimurium* DT104 strains inoculated into three ground pork products containing various fat contents and obtained D10 values for salmonellae from 0.42 to 0.62 kGy. The data prove that the content of fat had no effect on radiation resistance of salmonellae. The D10 values are similar to the values reported by Szczawińska [26] for *S. typhimurium* strains inoculated into poultry meat. Clavero et al. [27] subjected raw ground beef patties inoculated with mixture of serovars of *S. dublin*, *S. typhimurium*, and *S. enteritidis* to gamma irradiation (60°C) treatment. The influence of two levels of fat (8–14% (low fat) and 27–28% (high fat)) and temperature (frozen (–17 to –15°C) and refrigerated (3–5°C)) on the inactivation of pathogens by irradiation was investigated. D10 values for salmonellae in beef patties ranged from 0.618 to 0.800 kGy. The authors discovered that temperature did not have a significant effect when salmonellae were irradiated in high-fat ground beef.

D10 values for *Salmonella* spp. have been reported [28] to range from 0.38 to 0.77 kGy at 2°C in mechanically deboned chicken; sensitivity of *Salmonella* spp. to ionizing radiation has been found to be highly dependent on serovars. Similarly, the D10 values were reported by Szczawińska [26] for *S. typhimurium* strains inoculated into poultry meat, whereas a D10 value of 0.57 kGy has been observed for the pathogen in ground beef treated at 18–20°C [29].

In another work by Thayer et al. [30], *Musculus longissimus dorsi* from beef, pork, and lamb and turkey breast and leg meats were inoculated with *Salmonella* spp., and the gamma radiation resistance of the pathogens was determined at 5°C under identical conditions. The authors concluded that the *D*-value for a mixture of *Salmonella* spp. was significantly lower on pork than on beef, lamb, turkey breast, and turkey leg meats; however, all *D*-values were within expected ranges. Thayer et al. [31] studied the survival of salmonellae in vacuum-canned, commercial MDCM. The MDCM was challenged with *S. enteritidis* (ca 10⁴ CFU/g of meat) followed by irradiation to 0, 1.5, and 3.0 kGy and storage at 5°C for 0, 2, and 4 weeks. The researchers reported that the number of salmonellae in unirradiated MDCM decreased about one log cycle after 1 month of storage; however, in meat irradiated with 3.0 kGy dose the presence of this pathogen was not detected at the very beginning of storage. Thayer and Boyd also found that *S. typhimurium* was more resistant to gamma radiation when vacuum packaged than when air was present during irradiation [32]. The final equations predict a reduction in the number of surviving *Salmonella* in mechanically deboned chicken meat. If MDCM is irradiated at –20°C with a dose of 1.50 kGy in air then the expected reduction of this pathogen is 2.53 and 2.12 logs in vacuum. After 3.0 kGy dose, at –20°C in air the level of bacteria will be lower by 4.78 and 4.29 logs in vacuum [32].

Bacteria are more resistant when irradiated at frozen temperatures compared to chill or ambient temperatures; Szczawińska [26] reported that the mean D₁₀ value for 13 *Salmonella* strains irradiated in chicken meat using gamma rays at 4°C amounted to 0.575 kGy, whereas for samples irradiated in a frozen state (at –18°C) the mean D₁₀ value amounted to 0.687 kGy.

Gamma-irradiated broiler halves packed in polyethylene pouches with the dose of 2.5 kGy should ensure *Salmonella* reduction adequate to eliminate naturally occurring contamination. In frozen poultry meat, similar effects can be expected after a dose of 3.5 kGy [26]. In the same work, Szczawińska [26] discovered that the packaging material exerted a very strong effect on radiation resistance of *S. typhimurium*. Two strains of *S. typhimurium* were irradiated in ground chicken meat at temperatures +4 and -18°C. D10 values obtained for salmonellae irradiated at +4 and packed in PE pouches were 0.194 and 0.210 kGy, whereas D10 values obtained for salmonellae packed in PA/PE laminate pouches at the same temperature were 0.424 and 0.533 kGy. D10 values obtained for salmonellae irradiated at -18°C and packed in PE pouches were 0.412 and 0.633 kGy, whereas D10 values obtained for salmonellae packed in PA/PE laminate pouches at the same temperature were 0.538 and 0.721 kGy. Thus, the contribution of food-packaging material and packaging system is a very important issue in this technology. Irradiation was also combined with curing salts. The combined effects of 1-kGy irradiation dose and curing salts (NaNO₂ and NaCl) on the survival of *S. typhimurium*, *S. agona*, and *S. choleraesuis* in pork meat were studied by Szczawiński et al. [33]. Salmonellae were inoculated in ground *M. longissimus dorsi*, and irradiated at 1 kGy dose. The three experimental groups were designed. The meat was treated with 100 mg NaNO₂, 200 mg NaNO₂, and 200 mg NaNO₂ plus 3% NaCl. Meat samples were stored at 0–2°C for 3 weeks or at 20°C for 7 days. The authors reported that irradiation at 1 kGy dose reduced *Salmonella* number by 1.2–2 logs and that an additive effect of curing salts and irradiation was observed at low temperature of storage, and that synergistic effect of irradiation and curing salts was observed at temperature abuse [33].

Poultry, as already mentioned, as regards the radication, has been recognized as one of the best candidates for irradiation aiming a reduction or elimination of food-borne pathogenic bacteria such as *Salmonella* spp. and *Campylobacter* spp. Irradiation of poultry up to an overall average dose of 7 kGy was proposed by the Scientific Committee on Food [24] with the purpose to improve microbiological safety.

Salmonella caused 38.18%, the highest number of outbreaks and human cases among all causative agents according to data of EFSA for 2014 [34]. Raw poultry meat and poultry products are vehicles of those two food-borne pathogenic bacteria. In the EU, in 2013 [34], *Salmonella* was detected in 3.5% of the broiler meat. At retail, the overall proportion of *Salmonella*-positive samples was 7.5%, higher than at slaughterhouse (4.9%) and at the processing plant (2.6%) level [34]. Since December 2011, a *Salmonella* criterion for *S. enteritidis* and *S. typhimurium* in raw poultry entered into force [35].

In 2013, EFSA [34] reported that *Salmonella* was found in 0.3% of the 4776 samples of RTE broiler meat products tested at retail or at processing (0.1% of single samples and 1.9% of batches). Of the 2100 tested units of RTE products from turkey meat, only 0.1% in total were found to be *Salmonella*-positive [34].

Kudra et al. [36] studied the survival of *S. typhimurium* subjected to irradiation combined with high-CO₂ + CO MAP in chicken meat product. The authors did not find significant difference between D10 values for bacteria irradiated in vacuum (0.55 kGy) or in high-CO₂ + CO MAP (0.54 kGy). The dose of 1.5 kGy decreased the number of salmonellae by three logs. *Salmonella* presence was detected in both packaging systems during cold storage. During storage of this meat product at temperature abuse (25°C), *Salmonella* was able to grow in both packaging

systems. The authors concluded that low-dose irradiation is a suitable method for destruction of this pathogen; however, packaging system did not exert significant influence on *Salmonella* number during storage at low temperature. The authors concluded that if the initial contamination of these pathogens is high, cross-contamination of ready-to-eat food at temperature abuse of the product is likely to continue to be a food safety concern regardless of irradiation treatment doses or packaging treatments.

Szczawińska [26] reported that the mean D_{10} value for 13 *Salmonella* strains irradiated in chicken meat using gamma rays at 4°C amounted to 0.575 kGy, whereas for samples irradiated in a frozen state (at -18°C) the mean D_{10} value amounted to 0.687 kGy. Gamma-irradiated broiler halves packed in polyethylene pouches with the dose of 2.5 kGy should ensure *Salmonella* reduction adequate to eliminate naturally occurring contamination; in frozen poultry meat, similar effects can be expected after a dose of 3.5 kGy [26].

Nassar et al. [37] evaluated the survival of *Salmonella virchow* inoculated into raw chicken carcasses as a result of radiation treatment (dose range of 2–7 kGy) or disinfection with three chemical substances. The presence of salmonellae in chicken meat was not detected after 7 kGy dose; however, after chemical disinfection this pathogen was still present.

On the basis of the various published data, it seems that the dose up to 7 kGy for frozen poultry and about 3.5 kGy for unfrozen meat can be recommended to reduce the most radioresistant vegetative pathogenic bacteria by five logs [18].

Thayer et al. [38] compared gamma radiation resistance of a mixture of salmonellae (*S. dublin*, *S. enteritidis*, *S. newport*, *S. senftenberg*, and *S. typhimurium*) in the so-called “exotic” meats such as ground bison, ostrich, alligator, and caiman meats at 5°C. The type of meat did not significantly alter the radiation resistance of salmonellae, and the D -value of 0.53 ± 0.02 kGy for *Salmonella* spp. was obtained. In the conclusions, authors emphasized that the efficacy of the radiation treatment in elimination of *Salmonella* spp. in exotic meats and non-exotic meats (e.g., poultry) is similar, thus similar control measures can be applied to ensure exotic meat safety. When considering cooked chilled and other ready-to-eat poultry meat products, the food-borne pathogens of higher concern are represented by *L. monocytogenes* and *Salmonella* spp. Hence, stricter microbiological criteria for poultry meat products intended to be eaten cooked, as amended by the Commission Regulation (EU) No 365/2010, which enhance food safety, must be respected by EU members [39]. Another (EC) regulation [40], which lays down general rules for food business operators on the hygiene of foodstuffs, requires food business operators to comply with microbiological criteria for foodstuffs. Regulation (EC) No 853/2005 [41], which sets specific hygiene rules for foods of animal origin, also requires that food business operators ensure compliance with microbiological criteria.

Radiation sensitivity of *L. monocytogenes* was determined by many authors (i.e., for example, see Ref. [42]). Reported D_{10} values for *L. monocytogenes* in cooked turkey nuggets were about 0.70 kGy, making *L. monocytogenes* generally more radiation-resistant than *Campylobacter* and *Salmonella*. Taking into consideration similar radiation sensitivity of *L. monocytogenes* and *Salmonella* spp., it can be assumed that doses of ionizing radiation effective for the inactivation of *L. monocytogenes* will be sufficient to inactivate salmonellae.

2.2. Reducing of *Salmonella* spp. in ready-to-eat foods

Ready-to-eat foods deserve special interest. Very often, they contain not only cooked poultry or other meats and cooked seafoods but also raw meats which are consumed without heat treatment (e.g., “filet américain” composed of raw beef meat). Thus, those complex RTE foods may represent an individual specific hazard to consumers since they are often composed of a mixture of several types of ingredients. RTE foods vary by country and may include, for example, dried meat (beef jerky), uncooked and fermented minced meat products (salami), cooked offal or minced meat products (chicken liver pâté or luncheon sausage), and cooked whole meat products (ham) [43]. Gormley et al. [44] conducted a wide study on microbiological quality of ready-to-eat specialty meats (2359 samples of continental sausages, cured/fermented, and dried meats) and reported that 0.4% were unacceptable due to the presence of *Salmonella* spp. or *L. monocytogenes* (>10(2) CFU/g). These unacceptable meats were all prepacked prior to supply to retail premises indicating that contamination with bacterial pathogens occurred earlier in the production chain; the authors emphasize how important it is to prevent food contamination before final packaging and to control conditions of storage.

Song et al. [45] investigated the efficacy of radiation treatment and fumaric acid on the reduction of *L. monocytogenes* and *S. typhimurium* inoculated into sliced ham. The authors noted the decrease of number of listeriae and salmonellae by 2.42 and 3.78 logs, respectively, after irradiation of this ready-to-eat product while the decrease of only one log for both organisms was found after acid treatment.

The US Food and Drug Administration (FDA) is currently evaluating a petition to allow irradiation of RTE meats in the United States including deli turkey, ham, pastrami, beef Bologna, bacon bits, and pepperoni. The basis for the petition is data reported by Sommers and Mackay [46].

The authors observed in their study that irradiation of food-borne pathogenic bacteria with a dose of 3.75 kGy on ready-to-eat meats caused reduction of bacteria comparable to that obtained due to pasteurization, that is, minimum of five logs.

Sommers and Boyd [47] discovered that doses in the range of 2–4 kGy eliminate *Salmonella* spp. in many RTE foods.

The ability of ionizing radiation to inactivate *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, and *S. aureus* inoculated onto a frankfurter on a roll product containing the antimicrobials sodium diacetate and potassium lactate in the presence of an MA (100% N₂, 50% N₂ plus 50% CO₂, or 100% CO₂) was investigated. The authors reported that the radiation resistance (D10 values) for *Salmonella* in frankfurter on a roll product was from 0.61 to 0.71 kGy. MA had no effect on the radiation resistance of the pathogens. During a 2-week storage period under mild temperature abuse (10°C), both salmonellae and other pathogens were not able to proliferate on the frankfurter on a roll product, regardless of the MA used. Although the pathogens were unable to proliferate on the frankfurter on a roll product during the storage period, the application of a postpackaging intervention step was needed to actually inactivate the food-borne pathogens. The authors concluded that, when applied as a terminal intervention as part of a HACCP

plan, food irradiation could reduce the risk of food-borne pathogens on complex ready-to-eat foods such as sandwiches. It seems that intervention technologies including ionizing radiation, antimicrobials, and modified atmospheres (MAs) can be used to inhibit the growth of or inactivate food-borne pathogens on complex ready-to-eat foods such as sandwiches. Cárcel et al. [48] in their study elaborated mathematical model for the most efficient elimination of *Salmonella* spp. from two poultry products taking into consideration shelf life and sensory attributes. It was concluded that in the case of hamburgers, the optimum calculated dose was 2.04 kGy, which guaranteed the safety of the product and provided the best combination of sensory and instrumental attributes. As regards the steaks, the optimum assessed dose was 1.11 kGy, significantly lower than for hamburgers.

According to the data of a research project of a joint Food and Agriculture Organization/International Academy of Engineering (FAO/IAE), the application of ionizing radiation combined with other methods used for food preservation offers improved safety of many various prepared meals and longer shelf life [49].

Kang et al. [50] studied the efficacy of radiation treatment combined with leek (*Allium tuberosum* R.) extract on the survival of several food-borne pathogens inoculated in pork jerky. The authors used doses in the range of 0.5–4 kGy. The D_{10} -value for *S. typhimurium* irradiated with leek extract was 0.32 kGy and without this extract 0.39 kGy. The results prove that this combination strengthens both microbiological safety and shelf life of this meat.

2.3. Reducing *Salmonella* spp. in fish and shellfish

Raw fish and shellfish can be contaminated with pathogenic bacteria such as *Salmonella*, *Shigella*, *Vibrio parahaemolyticus*, *vulnificus*, *Vibrio cholerae*, *S. aureus*, and viruses.

According to the data delivered by the United States Department of Agriculture (USDA) [51], *Salmonella* was found in 21% of 153 aquaculture catfish collected from aquaculture ponds and retail markets. The U.S. Food and Drug Administration data from 1998 to 2004 on examination of seafood import refusal identified *Salmonella* contamination to be the most frequent violation in catfish (41.91% of violation categories). Hatha and Laksmanaperumalsamy [52] found *Salmonella* spp. in 14–25% of fish belonging to 18 families. On the basis of the data presented in the literature, along with outbreak data and FDA import refusal data, it can be concluded that the highest microbial hazard associated with catfish consumption is *Salmonella* spp. Raw finfish might contain *V. parahaemolyticus*, *Salmonella* spp., or *L. monocytogenes* [53]. According to the report of USDA, nontyphoidal *Salmonella* spp. in raw and RTE catfish are considered as higher priority microbial hazards [51]. In terms of risk assessment related to catfish consumption, USDA estimated that the mean reduction of *Salmonella* per catfish serving caused by frying is about two logs, and caused by baking is about three logs [51]. Thus, it seems that such reduction of *Salmonella* spp. number, taking into consideration that naturally contaminated foods contain usually low levels of salmonellae, may significantly lower the risk of food-borne disease due to consumption of contaminated catfish. These findings could partially explain the differences between a significant contamination of raw finfish by pathogenic bacteria and relatively small number of outbreaks in which etiologic agent is *Salmonella* spp.

The monthly data on import refusal published in the USA prove that 1/10 of the refused products are seafood products and that second in terms of rejection reason is the detection of *Salmonella* spp. [54]. Risk analysis conducted in New Zealand by Reed [55] for fillet meat of *Pangasius* spp. fish from Vietnam considered that contamination of fillets with water not of a suitable purity could result in the presence of exotic strains of *Corynebacterium diphtheriae*, *E. coli*, *Salmonella* spp., *V. cholerae*, and *Cryptosporidia* spp., which is a risk to human health. Shabarinath et al. [56] studied the prevalence of *Salmonella* in seafood samples by conventional culture and by a DNA-based molecular technique, polymerase chain reaction (PCR). Using PCR, which was considered to be better method, they isolated *Salmonella* spp. from over 50% of seafood samples collected from the southwest coast of India; 14 of 19 isolates belonged to serovar *Salmonella enterica* Weltevreden.

The FAO experts in their report, after thorough evaluation of *Salmonella* spp. problem related to seafood, concluded that good hygienic practices during aquaculture production and biosecurity measures can minimize but not eliminate *Salmonella* in products of aquaculture [57].

Among various seafood, shrimp as the largest single seafood commodity in value terms (at around 15% of the total value of internationally traded fishery products in 2012) mainly produced in developing countries such as South and East Asia and Latin America deserves special attention [58] particularly that the consumption of this commodity consumption has been trending upward.

Norhana et al. [59] in their comprehensive review paper on prevalence, persistence, and control of *Salmonella* and *Listeria* in shrimp and shrimp products indicated that the continued reporting of the presence of these bacteria in fresh and frozen shrimps, and even in the lightly preserved and ready-to-eat products, shows that the existing hygienic practices in fishery industry are insufficient to eliminate these pathogens which have been isolated from shrimps and shrimp products on a regular basis since the 1980s. Shrimp is frequently imported from tropical and subtropical areas and reports indicate that the product does not always meet the microbiological standards set for EU-producing countries or USA, because of either contaminated production sites or unhygienic processing conditions.

Salmonella bacteria are associated with pond water, sediment, and shrimp throughout the culture cycle, including the pre-stocking period, farming phase, and harvest. Untreated chicken manure used to fertilize ponds and droppings from aquatic birds are significant sources of *Salmonella*. The survival rate of the microorganism is enhanced by nutrients, manure, and feed present in the pond system and by the favorable interaction of various biological and physical factors [60]. Shrimps are usually eaten fully cooked. The major health hazards with these products are contamination during or after processing.

Pinu et al. [61] evaluated the microbiological condition of the frozen shrimps found in the local markets and departmental chain shops of Dhaka city. Pathogenic bacterial load was found greater in the samples of departmental shops rather than that of local markets. The researchers found *Salmonella* spp., *Vibrio* spp., and *Shigella* spp. in shrimps' samples and discovered that the samples collected from local markets and departmental shops were heavily contaminated and were of special concern for human consumption.

Asai et al. [62] reported that the examination of 353 samples of 29 types of seafood revealed that *S. enterica* serotype Weltevreden was isolated from two of 47 black tiger prawn samples. The contamination levels of *Salmonella* were in a range of <30–40 most probable number per 100 g. Asai concluded that these results indicate the possibility that shrimp and prawns contribute to food-borne infections.

In recent years, safety risks are associated to the consumption of raw or subjected to mild heat treatment fish and shellfish; molluscan shellfish (oysters, clams, mussels, and scallops) are often consumed whole and raw. Huss et al. [54] and Olgunoglu [63] in his comprehensive review on *Salmonella* in fish and fishery products show that the pathogens of concern in this seafood include both bacteria (e.g., *Vibrio* spp., *Salmonella* spp., *L. monocytogenes*, *Shigella* spp., *C. jejuni*), viruses (e.g., hepatitis A virus and norovirus), and parasites. Molluscan shellfish feed on phytoplankton and zooplankton. They are passive feeders that filter and concentrate pathogens present in harvest area. Their environment, particularly near-shore harvest water, is contaminated from sewage, which may contain pathogens from both human and animal fecal sources (e.g., *V. cholerae* O1 and O139, *Salmonella* spp.). Also, poor sanitary practices on the harvest vessel, poor aquacultural practices, and transportation can cause contamination of fishery products.

As observed in previous years, the food category with the highest level of non-compliance at processing was RTE fishery products (4.7% of single samples and 10.8% of batches), mainly in smoked fish [34].

Distribution of strong-evidence outbreaks by food vehicle in the EU in 2014 indicated that crustaceans, shellfish, molluscs, and products thereof were responsible for 8.1% of outbreaks with strong evidence (data from 592 outbreaks with strong evidence) [34]. Taking the above-mentioned data into consideration, health authorities in many countries including European Community emphasized that the increasing trend in raw fish consumption (sushi, sashimi, salmon, etc.) has been identified as a risk to human health. Oysters and mussels can cause food-borne illness. Consumer can contract food-borne salmonellosis due to consumption of raw oysters.

It is generally known that the best method of controlling pathogens is to use a postharvest treatment. Some treatments, such as thermal treatment, ionizing radiation, and high hydrostatic pressure processing, reduce the number of pathogenic microorganisms (bacteria and viruses) while the long-term freezing most widely used method of food preservation is mainly effective in controlling parasites.

Brands et al. [64] reported that *Salmonella* was isolated from oysters from each coast of the United States, and 7.4% of all oysters tested contained *Salmonella*. Isolation tended to be bay specific. The vast majority (78/101) of *Salmonella* isolates from oysters were *S. enterica* serovar Newport, a major human pathogen, confirming the human health hazard of raw oyster consumption. Bakr et al. [65] showed that out of the 150 seafood samples examined, collected from 11 localities in Alexandria, Egypt, *Salmonella* was isolated from 10% of samples (shrimp, oyster, and mussel). In 1986, the Scientific Committee for Foods [24] recommended that fish and shellfish could be irradiated at doses up to 3 kGy. In the United States, FDA has approved the use of ionizing radiation for the control of *V. parahaemolyticus* and *V. vulnificus* and other

food-borne pathogens in fresh or frozen molluscan shellfish. Irradiation of fresh and frozen molluscan shellfish may not exceed an absorbed dose of 5.5 kGy [53]. Also, FDA proposes radiation treatment for the control of food-borne bacteria in crustaceans with a dose of 6.0 kGy. The D10 values cited in the published literature for several *Salmonella* serotypes in grass prawns and shrimp homogenate ranged from 0.30 to 0.59 kGy. Thus, irradiation of crustaceans at a maximum absorbed dose of 6.0 kGy would be effective at controlling pertinent pathogens. The petitioner requested a maximum absorbed dose of 6.0 kGy to achieve a six-log reduction of *L. monocytogenes*. It can be expected that this dose should also eliminate majority of non-spore-forming pathogenic bacteria including *Salmonella*. Irradiation of fish and shellfish is intended, similarly like in the case of other foods to extend shelf life, reduce pathogen load, and inactivate parasites. Irradiation has been applied to fresh, frozen, as well as dried fish, fish products, and shellfish [18]. As for other foods, pathogenic bacteria are more resistant to irradiation in frozen state compared to chilled one. Most studies indicate that irradiation at doses recommended by the SCF (3 kGy) should yield two to five logs reduction of pathogenic, non-spore-forming bacteria for the majority of fish and fish products. Sommers and Rajkowski [66] determined the radiation D10 values for *Salmonella* inoculated onto seafood samples (scallops, lobster meat, blue crab, swordfish, octopus, and squid). The samples were frozen and irradiated in the frozen state (-20°C); D10 values for *Salmonella* ranged from 0.47 to 0.70 kGy. By contrast, the radiation D10 value for *Salmonella* suspended on frozen pork was 1.18 kGy. They concluded that radiation dose needed to inactivate these food-borne pathogens on frozen seafood is significantly lower than that for frozen meat or frozen vegetables. *Salmonella* spp. and other primary pathogens of concern can also be introduced after pasteurization. Some fishery products are cooked before they are packaged; therefore, they are at risk for recontamination between cooking and packaging (e.g., vacuum packaging, modified atmosphere packaging). Kamat and Thomas [67] evaluated the effect of fat content in fish on radiation sensitivity of *L. monocytogenes*, *Bacillus cereus*, *S. typhimurium*, and *Yersinia enterocolitica*. The radiation response of all those pathogens was examined in sardine with high fat and golden anchovy with low fat. The results clearly suggest that regardless of the level of lipid in fish, the application of a 3 kGy dose at refrigeration temperature would effectively decontaminate approximately 10^5 CFU g^{-1} of all the organisms tested, except spores of *B. cereus*. The authors concluded that the studies revealed a lack of influence of lipid levels in fish on radiation resistance of four food-borne bacterial pathogens.

Jakabi et al. [68] studied the survival of *S. enteritidis* and *S. infantis* inoculated into oysters and sensory properties as the result of irradiation with doses in the dose range of 0.5–3.0 kGy. The number of those both *Salmonella* populations decreased after a 3.0 kGy dose by five to six logs. The authors also discovered that oysters irradiated with the highest dose were still alive and concluded that a dose of 3.0 kGy could be considered effective in inactivating *Salmonella* in oysters without changing their odor, flavor, or appearance.

The SCF [24] recommended that shrimps could be irradiated at doses of 5 kGy which is considered to be an effective decontamination method. Ito et al. [69] reported that the dose of gamma irradiation necessary to reduce both *S. typhimurium* and *L. monocytogenes* in frozen shrimps at a level of below 10^{-4} per gram was about 3.5 kGy. Sinanoglou et al. [70] irradiated using a cobalt-60 gamma source frozen molluscs (squid, octopuses, and cuttlefish) and crustaceans (shrimp) with different doses. The authors noted the substantial decrease of mesophiles

number in shrimp irradiated with the dose of 2.5 kGy, whereas after the dose of 4.7 kGy the presence of those bacteria in squid was not detected. Shrimp is considered separately from fish and shellfish given that certain pathogens (i.e., *L. monocytogenes*) require doses about 3 kGy for several log₁₀ reduction. Sommers et al. [71] evaluated the effect of cryogenic freezing (−82°C, 3 min), and gamma irradiation on the survival of mixture of *Salmonella* spp. (*S. schwarzengrund*, *S. bahrenfeld*, *S. weltevreden*, and *S. panama* isolated from seafood, including shrimp), on raw frozen shrimp. D10 values for salmonellae irradiated in shrimp were about 0.56 kGy. The authors observed the decrease of *Salmonella* spp. number after cryogenic freezing and irradiation with a dose of 2.25 kGy by over five logs and that this effect persisted during 3 months storage at −20°C. The authors conclude that radiation treatment combined with cryogenic freezing offers big benefits in regard to frozen shrimp.

Nerkar and Bandekar [72] studied radiation resistance of *S. typhimurium* and *S. enteritidis* inoculated at 1×10^8 cells/ml in shrimp homogenate and they determined that the D10 value was in the range from 0.30 to 0.40 kGy. Finally, they concluded that a dose of 4.0 kGy could be used to completely eliminate *Salmonella* in frozen prepackaged shrimp.

Luo et al. [73] studied radioresistance of non-spore-forming and spore-forming pathogenic microorganisms inoculated into shelf-stable foods, semi-dried pork, and fish which have been vacuum-packaged. The water activity (aw) of semi-dried food products ranged between 0.930 and 0.940 for pork, and 0.852 and 0.895 for fish. The authors observed that *S. enteritidis* was eliminated at a dose of 2.5 kGy in semi-dried fish, and the minimum irradiation dose required to inactivate this pathogen in pork was 5 kGy.

2.4. Reducing *Salmonella* spp. in frog's legs

The skin of frogs and their internal organs are often contaminated with *Salmonella* spp. and other pathogens, such as *E. coli* and *S. aureus*. Although frog's legs are cooked before consumption, there is a risk for cross-contamination.

The highest radiation dose for frog's legs suggested by the Scientific Committee for Foods is 5 kGy [18]. The most important hazard arises from contamination with *Salmonella* and other fecal pathogens occurring in frog's legs at the time of deep-freezing. *E. coli* and *S. aureus* have been also found in frog's legs. Tambunan's [74] studies showed that irradiating frog legs artificially contaminated with *Salmonella* up to 10^6 /g before freezing a dose of 3 kGy and above resulted in no detection of the bacteria. If irradiation was carried out after freezing, a dose of 4 kGy and above has to be used. The latter procedure appears to be more feasible commercially than the former one. It was concluded that a combination of chlorination, freezing, and irradiation with a dose ranging from 3 to 6 kGy should provide sufficient conditions for the elimination of *Salmonella* in the product.

3. Concluding remarks

Ionizing radiation in industry can be used to reduce the level of *Salmonella* spp. in both raw and cooked meats, poultry, and seafood. This intervention technology can be regarded as a Critical Control Point in the HACCP plan. Irradiation treatment, applied as the final processing step,

seems to be of particular importance in the case of packed food products, including ready-to-eat food. In the USA, FDA [75] proposes radiation treatment with the maximum dose of 4.5 kGy for a variety of raw meats and meat products for the improvement of microbial safety and for shelf-life extension.

The data from literature prove that the *D*-values for *L. monocytogenes* are similar to those reported for *Salmonella* spp. irradiated under similar conditions. Thus, *Salmonella* spp. in meats, poultry, and fish and shellfish including ready-to-eat foods may be controlled by the same dose required for *L. monocytogenes*.

It should be noted, however, that dose range used for radication (2.5–10 kGy) is not sufficient to sterilize foods. Thus, all additional control measures (e.g., an unbroken cold chain, appropriate handling of raw meat, and procedures for cleaning disinfection and waste disposal, etc.) should maintain or even increase the beneficial effects of radiation treatment.

Referring to irradiation facilities, electron beams are much more useful for packs of relatively thin cooked, sliced meats, and other ready-to-eat products while gamma radiation is more suited for treating whole carcasses [76].

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